



# Carotenoid Measurement in Infant Formula Using a Validated Analytical Method



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## Abstract

**Background:** Studying the effects of dietary carotenoids in infants is complicated due to an absence of information on carotenoid concentrations in formula and due to naturally variable amounts found in human milk. Therefore, human milk and formula carotenoids must be quantified using a valid analytical method.

**Objective:** To accurately and reliably measure carotenoids in commercial infant formula using a previously validated carotenoid extraction method.

**Methods:** Beta-carotene, lutein, and lycopene were extracted according to a modified version of Schimpf, et al. (2018. *J AOAC Intl*, 101:1249-1252) and quantified by high-performance liquid chromatography-photodiode array detection. Accuracy and reliability were determined for a standardized infant formula reference material from the National Institute of Standards and Technology (NIST) (N=5 trials). Carotenoids were measured by the analysis of six commercial formulae (N=5 trials).

**Results:** Carotenoids could be extracted for accurate and reliable quantitation of standardized NIST formula, with average relative standard deviations of 11%, 3%, and 14% for beta-carotene, lutein, and lycopene, respectively, and measured carotenoid concentration ranges overlapped with certified ranges. Lutein was most abundant across 5 brands of Manufacturer A’s formula, followed by lycopene and beta-carotene. Only beta-carotene was detected in manufacturer B’s formula.

**Conclusions:** The method reliably and accurately extracts carotenoids from standardized infant formula reference material. Carotenoids in the majority of commercial formulae correspond with manufacturer-provided ingredients lists.

## Background

- Carotenoids are a class of antioxidant compounds naturally found in fruits and vegetables, and are associated with a number of health benefits across the lifespan.
- Carotenoids are also found in human milk and are added to infant formula by formula manufacturers.
- Carotenoid concentrations in commercial formula are not available from the USDA Nutrient Database or nutritional labels, impairing our ability to quantify infant carotenoid intake from formula.
- Determining infants’ carotenoid intake can better help researchers understand the various roles carotenoids play in human health

## Objective

- We aim to:
  - Extract and quantify carotenoids from major manufacturers’ branded infant formula using a modified version of the published method by Schimpf, et al. (1).
  - Monitor if the modified version of the previously published method reliably and accurately extracts carotenoids from a standardized infant formula reference material.

## Hypothesis

- The extraction method will permit the accurate measurement of carotenoid concentration from the standardized infant formula reference material.
- Detection and quantitation of carotenoids in commercial formula will be consistent with the carotenoids listed on the ingredients lists where we will detect:
  - Beta-carotene, lutein, and lycopene in Manufacturer A’s formula
  - Beta-carotene and lycopene in Manufacturer B’s formula

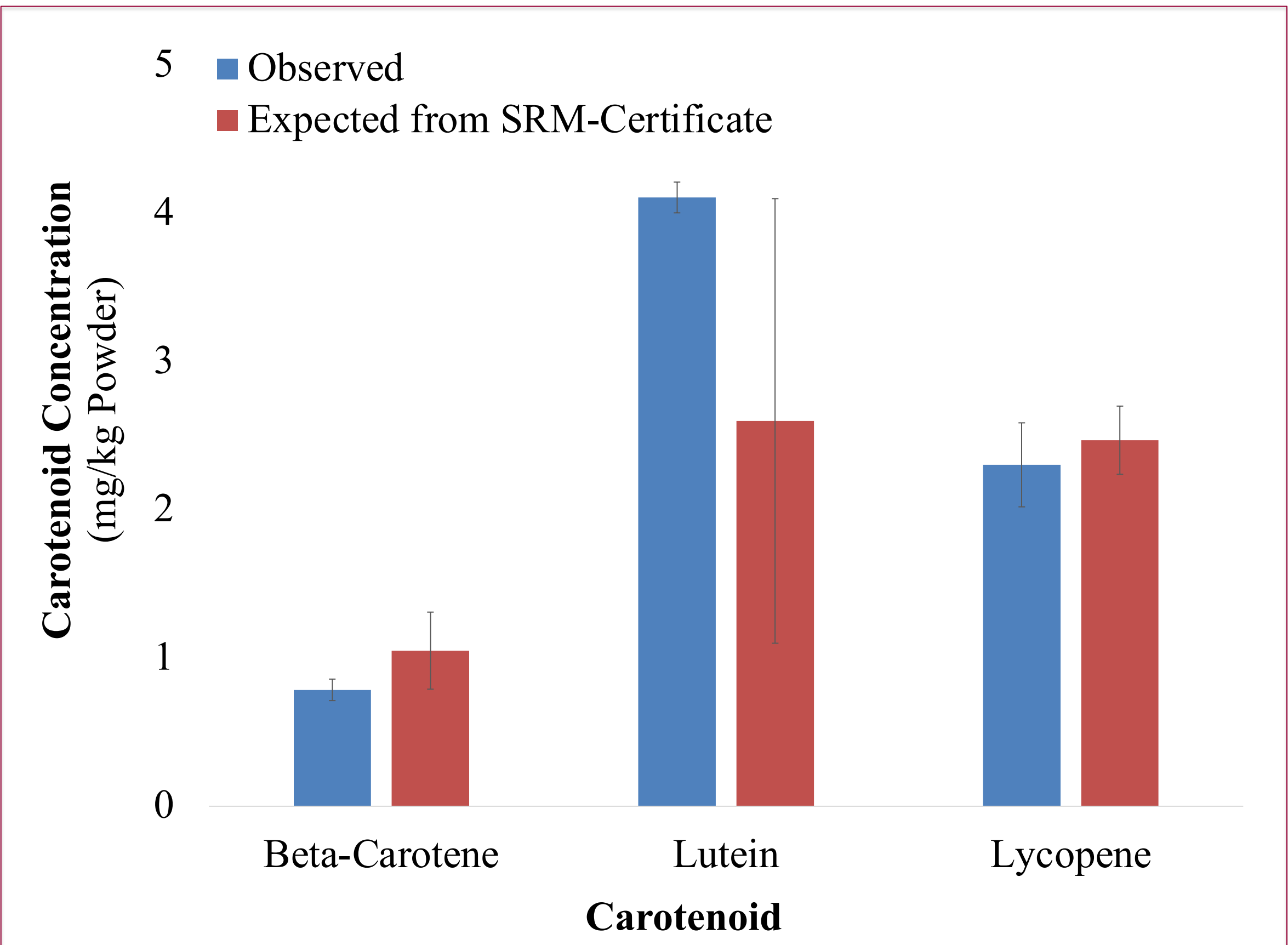
## Study Design

- Carotenoid determination:** ability to measure carotenoids in 6 commercial formula products produced by two major manufacturers.
  - Formulae were selected on the basis of market popularity obtained from market research firms (3) and use by Texas WIC
  - Testing of commercial formula was conducted using samples prepared in duplicate and analysed over N = 5 trials
- Validity determination:** ability to obtain carotenoid concentrations for a standardized infant formula reference material available from the National Institutes of Standards and Technology:
  - Overlap with the 95% C.I.’s provided by NIST indicating accuracy
  - With low relative standard deviations (RSDs) indicating reliability
  - N = 5 trials, 2 reps/trial

## Methods

- Following formula dissolution in water and heated saponification with potassium hydroxide, carotenoids were twice extracted from infant formula using a biphasic, organic and aqueous solvent based approach (1)
- Samples were analyzed by high-performance liquid chromatography-photodiode array detector with the following parameters:
  - Carotenoids were separated on a C30 column (3.0 x 150 mm, 3 um, YMC) using a modified gradient method of methanol, methyl *tert*-butyl ether, and aqueous ammonium acetate (2) at 0.34 mL/min
  - Carotenoids were quantified using external calibration standards and extraction efficiency was corrected relative to an internal recovery standard of apo-8’-carotenal

## Results



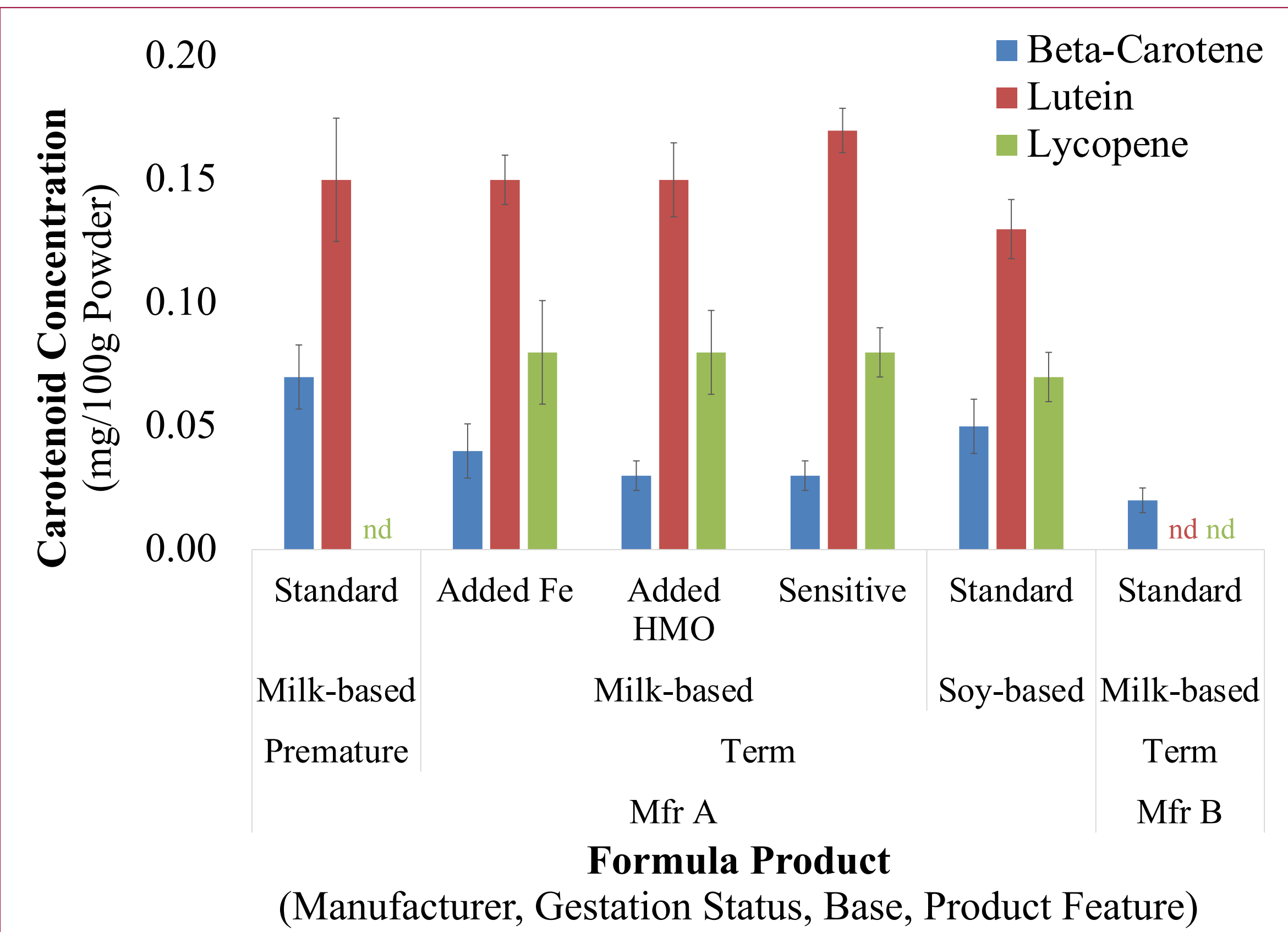
**Figure 1.** Observed vs. certified carotenoid concentrations (mg/kg) in NIST SRM-1869 infant formula. Blue bars represent the observed mean  $\pm$  95% C.I. Red bars represent the certified mean  $\pm$  95% C.I. N = 5 trials, 2 replicates/trial.

## Results

**Table 1.** Relative standard deviations of carotenoid concentrations measured in standardized infant formula reference material.<sup>1</sup>

	Beta-Carotene	Lutein	Lycopene
Average RSD	11%	3%	14%

<sup>1</sup> N = 5 trials, 2 reps/trial



**Figure 2.** Concentrations of carotenoids (mg/100g Powder) in 6 commercial formulae. N = 5 trials, 2 reps/trial. “nd” refers to not detected in analysis.

## Conclusion

- Overlap between observed and certified standard infant formula reference values and low variability suggests this extraction method is both accurate and reliable.
- Carotenoids were fairly consistent across Manufacturer A’s infant formula and correlated with official ingredients list.
- Only beta-carotene was present in Manufacturer B’s infant formula.
- Exact commercial formula concentrations can be used to assist researchers in understanding the carotenoid exposure of infants.
- This validated method can be implemented for studying the relationship between infant dietary carotenoid intake and health.

## Acknowledgements

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